

LISTING OF CLAIMS

1-185. (Cancelled)

186. (Currently Amended)

A method of artificial insemination comprising the steps of:

- a. collecting sperm cells from a male of a nonhuman mammal species;
- b. determining the sex characteristic of a plurality of said sperm cells;
- c. sorting said sperm cells according to the determination of their sex characteristic at a rate of at least 1200 ~~sorts second~~ sorts per second;
- d. establishing an artificial insemination sample from said sorted sperm cells;
- e. freezing said artificial insemination sample established from said sorted sperm cells;
- f. thawing said artificial insemination sample established from said sorted sperm cells;
- g. inserting at least a portion of said thawed artificial insemination sample established from said sorted sperm cells into a female of ~~said nonhuman a~~ nonhuman mammal species;
- h. fertilizing at least one egg within said female of ~~said nonhuman a~~ nonhuman mammal species.

187. (Previously Presented)

A method of artificial insemination as described in claim 186 wherein said step of collecting comprises the step of collecting sperm cells from a male of a nonhuman mammal species selected from the group consisting of bovines and equines.

188. (Previously Presented)

A method of artificial insemination as described in claim 186 or 187 wherein said steps of inserting and fertilizing are each accomplished in a field environment.

189. (Currently Amended)

- A method of artificial insemination as described in claim 188 wherein said steps of inserting and fertilizing in a field environment comprise the steps of repetitively inserting a significant number of said thawed artificial insemination samples into a significant number of said females of ~~said nonhuman~~ a nonhuman mammal species in rapid succession and in farm or ranch conditions.
190. (Currently Amended)
- A method of artificial insemination as described in claim 186 wherein said female of ~~said nonhuman~~ a nonhuman mammal species has uterine horns and wherein said step of inserting comprises the step of inserting said thawed artificial insemination sample both ipsi- and contra-lateral within said uterine horns of said female of ~~said nonhuman~~ a nonhuman mammal species.
191. (Currently Amended)
- A method of artificial insemination as described in claim 186 or 187 wherein said female of ~~said nonhuman~~ a nonhuman mammal species has at least one uterine horn and wherein said step of inserting comprises the step of inserting said thawed artificial insemination sample within said uterine horn.
192. (Currently Amended)
- A method of artificial insemination as described in claim 191 wherein said step of inserting within said uterine horn further comprises the step of inserting said thawed artificial insemination sample deep within said uterine horn.
193. (Currently Amended)
- A method of artificial insemination as described in claim 191 wherein said step of inserting within said uterine horn further comprises the step of inserting said thawed artificial insemination sample within said uterine horn through the use of embryo transfer equipment.
194. (Currently Amended)

A method of artificial insemination as described in claim 192 wherein said step of inserting deep within said uterine horn further comprises the step of inserting said thawed artificial insemination sample deep within said uterine horn through the use of embryo transfer equipment.

195. (Cancelled)

196. (Currently Amended)

A method of artificial insemination as described in claim 186 wherein said step of inserting comprises the step of inserting said thawed artificial insemination sample not later than about seventeen hours from said step of establishing said artificial insemination sample.

197. (Currently Amended)

A method of artificial insemination as described in claim 186 wherein said step of inserting comprises the step of inserting said thawed artificial insemination sample not later than about ten hours from said step of establishing said artificial insemination sample.

198. (Cancelled)

199. (Currently Amended)

A method of artificial insemination as described in claim 186 wherein said steps of determining the sex characteristic of a plurality of said sperm cells and sorting said sperm cells according to the determination of their sex characteristic comprise the steps of:

- a. providing a flow cytometer having a cell source which supplies sperm cells to be sorted;
- b. ~~establishing a cell source which supplies sperm cells to be sorted;~~
- c. ~~chemically~~

- b. ~~chemically~~ coordinating a sheath fluid to create a sheath fluid environment for said sperm cells which is coordinated with both a pre-sort and a post-sort sperm cell fluid environment;
- d. ~~sensing~~
- c. ~~identifying a property sex characteristic~~ of said sperm cells;
- e. ~~discriminating~~
- d. ~~discriminating~~ between said sperm cells having ~~a desired~~ said desired sex characteristic; and
- f. ~~collecting~~
- e. ~~collecting~~ said sperm cells having ~~the desired~~ said desired sex characteristic.

200. (Currently Amended)

A method of artificial insemination as described in claim 186 wherein said steps of determining the sex characteristic of a plurality of said sperm cells and sorting said sperm cells according to the determination of their sex characteristic comprise the steps of:

- a. providing a flow cytometer having a cell source which supplies bovine sperm cells to be sorted;
- b. ~~establishing a cell source which supplies sperm cells to be sorted~~;
- e. ~~establishing~~
- b. ~~establishing~~ a sheath fluid for said bovine sperm cells which contains about 2.9% sodium citrate;
- d. ~~sensing~~
- c. ~~identifying a property sex characteristic~~ of said bovine sperm cells;
- e. ~~discriminating~~
- d. ~~discriminating~~ between said bovine sperm cells having ~~a desired~~ said desired sex characteristic; and
- f. ~~collecting~~
- e. ~~collecting~~ said bovine sperm cells having ~~the desired~~ said desired sex characteristic.

201. (Currently Amended)

A method of artificial insemination as described in claim 186 wherein said steps of determining the sex characteristic of a plurality of said sperm cells and sorting said sperm cells according to the determination of their sex characteristic comprise the steps of:

- a. providing a flow cytometer having a cell source which supplies equine sperm cells to be sorted;
- b. ~~establishing a cell source which supplies sperm cells to be sorted;~~
- e. ~~establishing~~
- b. ~~establishing~~ a sheath fluid for said equine sperm cells which contains a hepes buffered medium;
- d. ~~sensing~~
- c. ~~identifying a property~~ sex characteristic of said equine sperm cells;
- e. ~~discriminating~~
- d. ~~discriminating~~ between said equine sperm cells having ~~a desired~~ said desired sex characteristic; and
- f. ~~collecting~~
- e. collecting said equine sperm cells having ~~the desired~~ said desired sex characteristic.

202. (Currently Amended)

A method of artificial insemination as described in claim 186 wherein said steps of determining the sex characteristic of a plurality of said sperm cells and sorting said sperm cells according to the determination of their sex characteristic comprise the steps of:

- a. providing a flow cytometer having a cell source which supplies sperm cells to be sorted;
- b. ~~establishing a cell source which supplies sperm cells to be sorted;~~
- e. ~~establishing~~
- b. ~~establishing~~ a sheath fluid for said sperm cells;

- d. ~~sensing~~
- c. ~~identifying a property sex characteristic of said sperm cells;~~
- e. ~~discriminating~~
- d. ~~discriminating between said sperm cells having a desired said desired sex characteristic; and~~
- f. ~~collecting~~
- e. ~~collecting said sperm cells having the desired said desired sex characteristic while cushioning said sperm cells from impact with a collector.~~

203. (Currently Amended)

A method of artificial insemination as described in claim 186 wherein said steps of determining the sex characteristic of a plurality of said sperm cells and sorting said sperm cells according to the determination of their sex characteristic comprise the steps of:

- a. providing a flow cytometer having a cell source which supplies sperm cells to be sorted;
- b. ~~establishing a cell source which supplies sperm cells to be sorted;~~
- e. ~~establishing~~
- b. ~~establishing a sheath fluid for said sperm cells;~~
- d. ~~sensing~~
- c. ~~identifying a property sex characteristic of said sperm cells;~~
- e. ~~discriminating~~
- d. ~~discriminating between said sperm cells having a desired said desired sex characteristic; and~~
- f. ~~collecting~~
- e. ~~collecting said sperm cells having the desired said desired sex characteristic in a citrate collection fluid containing about six percent egg yolk.~~

204. (Cancelled)

205. (Previously Presented)

A method of artificial insemination as described in claim 186 and further comprising the step of using an ovulatory pharmaceutical to cause multiple eggs to be produced and wherein said step of fertilizing comprises the step of fertilizing a plurality of said eggs to produce multiple embryos, wherein said ovulatory pharmaceutical is injected in half day increments between any of days 2 and 18 of the estrus cycle.

206. (Previously Presented)

A method of artificial insemination as described in claim 205 wherein said step of using an ovulatory pharmaceutical to cause multiple eggs to be produced comprises the step of injecting a dosage of follicle stimulating hormone.

207. (Previously Presented)

A method of artificial insemination as described in claim 206 wherein said step of injecting said dosage of follicle stimulating hormone in approximately half day increments comprises a dosage level of 6, 6, 4, 4, 2, 2, 2, and 2 mg between days 9 and 12 inclusive of the estrus cycle and further comprising the step of injecting 25 and 12.5 mg of prostaglandin F-2-alpha on the sixth and seventh dosages, respectively, of said follicle stimulating hormone.

208. (Previously Presented)

A method of artificial insemination as described in claim 186 wherein said steps of determining the sex characteristic of a plurality of said sperm cells and sorting said sperm cells according to the determination of their sex characteristic further comprise the steps of:

- a. staining said sperm cells with at least about 38 micro-molar content of stain; and
- b. concentrating said sorted sperm cells of said male nonhuman mammal.

209. (Cancelled)

210. (Previously Presented)

A method of artificial insemination as described in claim 186 further comprising the step of chemically coordinating a sheath fluid environment for said sperm cells which is coordinated with both a pre-sort and a post-sort sperm cell fluid environment.

211. (Previously Presented)

A method of artificial insemination as described in claim 210 wherein said step of chemically coordinating a sheath fluid to create a sheath fluid environment for said cells which is coordinated with both a pre-sort and a post-sort sperm cell fluid environment comprises the step of establishing a cell source which supplies bovine sperm cells and the step of establishing a sheath fluid which contains about 2.9% sodium citrate.

212. (Previously Presented)

A method of artificial insemination as described in claim 210 wherein said step of chemically coordinating a sheath fluid to create a sheath fluid environment for said sperm cells which is coordinated with both a pre-sort and a post-sort sperm cell fluid environment comprises the step of establishing a cell source which supplies equine sperm cells and the step of establishing a sheath fluid which contains a hepes buffered medium.

213. (Previously Presented)

A method of artificial insemination as described in claim 186 wherein said step of sorting said sperm cells according to the determination of their sex characteristic further comprises the step of cushioning said cells from impact with a collector.

214. (Previously Presented)

A method of artificial insemination as described in claim 213 wherein said step of cushioning said cells comprises the step of providing a collection container having a diameter of at least fifteen millimeters.

215. (Previously Presented)

A method of artificial insemination as described in claim 213 wherein said step of cushioning said cells comprises the step of avoiding impact of said cells with said collector.

216. (Previously Presented)

A method of artificial insemination as described in claim 213 wherein said step of cushioning said cells comprises the step of providing a collection container having stream matched physical characteristics.

217-218.(Cancelled)

219. (Currently Amended)

A method of artificial insemination as described in claim 186 wherein said step of establishing an artificial insemination sample comprises the step of establishing an artificial insemination sample having a ~~low number~~ number of said sperm cells ~~relative to a typical artificial insemination sample~~ selected from the group consisting of: a bovine insemination sample of no more than one hundred thousand sperm cells, a bovine insemination sample of no more than two hundred fifty thousand sperm cells, a bovine insemination sample of no more than three hundred thousand sperm cells, a bovine insemination sample of no more than five hundred thousand sperm cells, a bovine insemination sample of no more than one million sperm cells, and a bovine insemination sample of no more than five million sperm cells.

220. (Currently Amended)

A method of artificial insemination as described in claim 186 wherein said step of establishing an artificial insemination sample comprises the step of establishing an artificial insemination sample having a ~~low-number~~ number of said sperm cells ~~relative to a typical artificial insemination sample~~ selected from the group consisting of: an equine insemination sample of no more than one million sperm cells, an equine insemination sample of no more than five million sperm cells, an equine insemination sample of no more than ten million sperm cells, and an equine insemination sample of no more than twenty-five million sperm cells.

221. (New)

A method of bovine artificial insemination comprising the steps of:

- a. collecting sperm cells from a male bovine;
- b. staining said sperm cells with 38 micro-molar Hoechst 33342;
- c. determining the sex characteristic of a plurality of said stained sperm cells with a flow cytometer;
- d. sorting said stained sperm cells according to the determination of their sex characteristic at a rate of at least 500 sorts per second with a flow cytometer operating at at least 50 psi;
- e. concentrating said sperm cells following said step of sorting;
- f. establishing an artificial insemination sample having no more than about 350,000 of said sorted sperm cells;
- g. inserting said artificial insemination sample into a female bovine;
- h. fertilizing at least one egg within said female bovine at success levels statistically comparable to an unsorted artificial insemination sample having about an equal number of sperm cells.

222. (New)

A method of bovine artificial insemination as described in claim 221 wherein said step of inserting said artificial insemination sample into a female bovine comprises the step of inserting said artificial insemination sample into a female bovine no more than about 5 hours following said step of sorting.

223. (New)

A method of bovine artificial insemination as described in claim 221 wherein said step of inserting said artificial insemination sample into a female bovine comprises the step of inserting said artificial insemination sample into a female bovine no more than about 9 hours following said step of sorting.

224. (New)

A method of bovine artificial insemination as described in claim 221 wherein said step of inserting said artificial insemination sample into a female bovine comprises the step of inserting one-half of said artificial insemination sample into each uterine horn of said female bovine.

225. (New)

A method of bovine artificial insemination as described in claim 221 wherein said step of inserting said artificial insemination sample into a female bovine comprises the step of inserting said artificial insemination sample into a female bovine no more than about 6 hours following observed standing estrus of said female bovine.

226. (New)

A method of bovine artificial insemination as described in claim 221 wherein said step of inserting said artificial insemination sample into a female bovine comprises the step of inserting said artificial insemination sample into a female bovine no more than about 26 hours following observed standing estrus of said female bovine.

227. (New)

A method of bovine artificial insemination as described in claim 221 further comprising the step of cooling said artificial insemination sample to at least 5 degrees Celsius prior to said step of inserting.